



US005788958A

United States Patent [19]

Dewhirst et al.

[11] **Patent Number:** 5,788,958[45] **Date of Patent:** Aug. 4, 1998[54] **METHODS FOR IMPROVING
THERAPEUTIC EFFECTIVENESS OF
AGENTS FOR THE TREATMENT OF SOLID
TUMORS AND OTHER DISORDERS**[75] Inventors: **Mark W. Dewhirst**, Chapel Hill;
Robert E. Meyer, Cary; **Joseph
Bonaventura**, Beaufort, all of N.C.;
Joseph DeAngelo, Hamtramck, Mich.[73] Assignees: **Duke University**; **Apex Bioscience,
Inc.**, both of Durham; **North Carolina
State University**, Raleigh, all of N.C.[21] Appl. No.: **709,938**[22] Filed: **Sep. 6, 1996****Related U.S. Application Data**[60] Division of Ser. No. 246,882, May 20, 1994, Pat. No.
5,554,638, which is a continuation-in-part of Ser. No.
66,756, May 24, 1993, Pat. No. 5,612,310.[51] **Int. Cl.**⁶ **A61K 31/77**; A61K 38/16;
A01N 37/24; C07C 205/13[52] **U.S. Cl.** **424/78.38**; 424/85.2; 514/6;
514/12; 514/564; 514/561; 514/723; 562/560;
562/553[58] **Field of Search** 424/78.38, 85.2;
514/6, 12, 564, 565, 561, 723, 560, 930;
562/560, 553[56] **References Cited****U.S. PATENT DOCUMENTS**

4,001,401 1/1977 Bensen et al. .
 4,061,736 12/1977 Morris et al. .
 4,301,144 11/1981 Iwashita .
 4,321,259 3/1982 Nicolau .
 4,377,512 3/1983 Ajisaka .
 4,412,989 11/1983 Iwashita .
 4,473,563 9/1984 Nicolau .
 4,584,130 4/1986 Bucci et al. .
 4,598,064 7/1986 Walder .
 4,650,786 3/1987 Wong .
 4,670,417 6/1987 Iwasaki .
 4,710,488 12/1987 Wong .
 4,812,449 3/1989 Rideout .
 5,028,588 7/1991 Hoffman et al. .
 5,266,594 11/1993 Dawson et al. .
 5,273,875 12/1993 Griffith .
 5,296,466 3/1994 Kilbourn et al. .
 5,298,490 3/1994 Heavner et al. .
 5,298,506 3/1994 Stamler et al. .
 5,312,835 5/1994 Kilbourn et al. .
 5,317,040 5/1994 Goldman .
 5,334,380 8/1994 Kilbourn et al. .

FOREIGN PATENT DOCUMENTS

WO 88/03408 5/1988 WIPO .
 WO 90/13645 11/1990 WIPO .
 WO 93/08831 5/1993 WIPO .

OTHER PUBLICATIONS

Andrade et al., "Inhibitors of nitric oxide synthase selectively reduce flow i tumor-associated neovasculature", Br. J. Pharmacol. 107:1092-1095, 1992.

Babbs and DeWitt, "Physical principles of local heat therapy for cancer", Med. Instrum. 15:367-373, 1981.

Botstein and Shortle, "Strategies and applications of in vitro mutagenesis", Science 229:1193-1201, 1985.

Brown and Koong, "Therapeutic advantage of hypoxic cells in tumors: a theoretical study", J. Natl. Cancer Inst. 83:178-185, 1991.

Caruthers et al., "New methods for synthesizing deoxyoligonucleotides", Genetic Engineering, J.K. Setlow and A. Hollaender eds., Plenum Press, New York, vol. 4, pp. 1-17, 1982.

Chapman et al., "Keynote address: cellular reduction of nitroimidazole drugs: potential for selective chemotherapy and diagnosis of hypoxic cells", Int. J. Rad. Oncology, Biol. Phys. 16:911-917, 1987.

Collman et al., "Picket fence porphyrins. Synthetic models for oxygen binding proteins", J. Am. Chem. Soc. 97:1427-1439, 1975.

De Venuto et al., "Appraisal of hemoglobin solution as a blood substitute", Surgery Gynecology and Obstetrics 149:417-436, 1979.

Dewhirst et al., "The use of hydralazine to manipulate tumour temperatures during hyperthermia", Int. J. Hyperthermia 6:971-983, 1990.

Feola et al., "Developemt of a bovine stroma-free hemoglobin solution as a blood substitute", Surgery Gynecology and Obstetrics 157:399-408, 1983.

Froehler, "Synthesis of DNA via deoxynucleotide H-phosphonate intermediates", Nucl. Acids. Res. 14:5399-5407, 1986.

Hahn and Shiu, "Protein synthesis, thermotolerance and step down heating", Int. J. Radiat. Oncol. Biol. Phys., 11:159-164, 1985.

(List continued on next page.)

Primary Examiner—Johann Richhter*Assistant Examiner*—Ebenezer Sackey*Attorney, Agent, or Firm*—Pennic & Edmonds LLP

[57]

ABSTRACT

The present invention is directed to the use of an inhibitor of NO activity, such as a nitric oxide scavenger or an NO synthase inhibitor, as an antitumor therapy to reduce tumor blood flow and oxygenation. The invention is also directed to administration of a nitric oxide scavenger or a nitric oxide synthase inhibitor to enhance the effectiveness of tumor therapy with hypoxic or acidic chemotherapeutic agents or hyperthermia. The invention is also directed to the administration of a nitric oxide synthase substrate to a subject previously administered a nitric oxide synthase inhibitor, in order to selectively inhibit tumor perfusion. In a specific example, administration of cell free hemoglobin, a nitric oxide scavenger, in conjunction with mitomycin C, a hypoxic cytotoxin, results in a significant delay in tumor growth of a human tumor xenograft in a mouse compared to mitomycin C alone. In another example, the administration of an inhibitor of nitric oxide synthase followed by the administration of a substrate of the enzyme causes a specific irreversible reduction of tumor blood flow, while normal blood flow is restored.

6 Claims, 14 Drawing Sheets